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Review

Strategies for Generating Human Pluripotent Stem Cell-Derived-Organoid Culture for Disease Modelling, Drug Screening, and Regenerative Therapy

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Abstract: Human pluripotent stem cells (hPSCs) have become a powerful tool to generate various kinds of cell types comprising the human body. Recently, organoid technology emerged as a platform to build a physiologically relevant tissue-like structure from the PSCs, which provides a more relevant three-dimensional microenvironment to the actual human body than the conventional monolayer culture system for transplantation, disease modeling, and drug development. Although it holds so many advantages, the organoid culture system still has various problems related to culture methods, which became a challenge to get similar physiological properties to their original tissue counterparts. Here, we discuss the current development of organoid culture methods, including the problem that may arise from the currently available culture systems as well as the possible approach to overcoming the current limitation and improving their optimum utilization for translational application purposes.

Keywords: hPSCs derived-organoids; Culture strategy; Disease modeling; Drug screening; Regenerative therapy

1. Introduction

Animal model is a routine platform to understand many biological processes related to human disease. Although it can partially represent several biological processes of human physiological and pathological conditions, this model can not accurately provide fully similar features of human tissue physiology and disease mechanism due to the interspecies genomic divergence which impacts various complex cellular activities [1], [2]. In actual translational applications, these possible differences may reduce the accuracy, potentially contributing to the low success rate in clinical trials of newly developed drugs [3]. On the other side, the usage of two-dimensional human cell culture did not completely represent the actual human tissue function and architecture[4].

Organoids are three-dimensional miniature organ-like structures that recapitulate physiologically relevant tissue function through self-organization in vitro. The generation of human pluripotent stem cells (hPSCs) derived-organoid can facilitate the opportunity to improve the current limitation in disease modeling or drug screening platforms, as well as open a new insight for their application in regenerative therapy [3]. This microtissue like-structure can be generated from healthy or diseased patients which may represent specific cellular and physiological conditions for personalized medicine application. The development of human-induced pluripotent stem cells (hiPSCs) enables various studies to be more experimentally accessible with fewer ethical constraints due to the usage of

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human embryonic stem cells (hESCs) that require a sacrificial of the human embryo. Moreover, this technology can be applied to generate patient-specific-hiPSCs-derived-organoids for a better personalized disease modeling [5], drug screening [6], or even regenerative therapy [7].

Currently, the hPSCs-derived organoid production mostly involved a complex multistep protocol during their differentiation and maturation process, which makes the production of organoids become technically challenging. Various techniques have been developed to produce the organoids from PSCs. However, some important factors related to the organoid culture system still need to be considered to achieve an optimum culture condition for each specific translational purpose. In this review, we highlight the currently available methodology to generate the organoids from pluripotent stem cells as well as the current challenge and possible alternatives to improve the generation of hPSCs derived-organoid.

2. The Features of hPSCs derived-organoids

Organoids became a promising research tool to elucidate the mechanism of various diseases and became a good platform for testing the effect of substance exposure, providing a precise preclinical setting [8]. The concept of organoid transplantation has also emerged as a future insight in aiming the alternative for partial tissue replacement for regeneration [9]. Nevertheless, the current culture methodology still needs to be improved to realize the reliable translational applications of hPSCs-derived-organoids.

At present, numerous studies of hPSCs-derived-organoids represent a broad spectrum of specific organ types for different applications (Table 1). The 3D organoid exhibits better crosstalk between the different cells inside its structure, which is can not be achieved by conventional monolayer culture. Therefore, these 3D "miniaturized organs" possess a decent capability to become a model with more physiologically relevant and predictive than monolayer culture, which reflects the cellular interactions between different cells within an organ during development, healthy, as well as disease conditions.

Generally, the organoid needs to meet some criteria which confirm the authenticity with the original organ [10]. Firstly, organoid should be a three-dimensional (3D) structure consisted of multiple cell types that retain the identity of the specific organ; Secondly, this 3D structure formed by self-organization according to the similar intrinsic organization principles of the organ; and third, the organoid should recapitulate the key features represent functional capability the actual organ, both in terms of structure and physiological activity [11], [12].

3. Culture Strategy to Generate hPSCs derived-Organoids

Currently, several methods have been developed to generate a miniaturized tissue-like organoid consisting of their complex functional cellular components from hPSCs, including human embryonic stem cells (hESCs) and human-induced pluripotent stem cells (hiPSCs). The differentiation process involved engineering and manipulation using a stepwise treatment using combined factors to direct the hPSC cell fates. This step can be improved by creating an in vitro culture environmental condition to induce a subsequent organ-specific lineage differentiation using a different culture platform. Depending on the applications and expected organoid type, several factors need to be considered when choosing a culture strategy to get the best hPSCs derived-organoid production outcomes (Table 2).

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Table 1. Selected study of hPSCs derived organoids and their targeted applications

Organoid type	PSCs source	Culture method	Targeted application	Results	Ref
Brain organoids	hiPSCs	Static 96-well V-bottom plates	Disease modeling	The cerebral organoid has successfully delineated defects in human lissencephaly caused by deletions of the distal tip of chromosome	[13]
	hiPSCs	Static ultra-low attachment 96 well plate and dynamic 24 well plates	Disease modeling	Human cerebral organoids establish differential effects in Neurofibromatosis type 1 (NF1) developmental disorder	[14]–[16]
	hiPSCs	Static culture low-adhesion 6-cm plates followed by Matrigel embedded	Disease modeling	This patient-specific forebrain organoids were utilized to investigate the pathological changes associated with Miller-Dieker syndrome (MDS) caused by chromosomal deletion	[5]
	hESCs and hiPSCs	ultra-low-attachment 96-well plates	Disease modeling	hPSCs derived-microglia is introduced to hPSCs derived-cerebral organoids. The microglia reflect the innate immune response system in the central nervous system which showed a phagocytic activity during Zika virus infection.	[17]
	hiPSCs	Miniaturized spinning bioreactor	Disease modeling and developmental study	Resulted brain organoids were successfully recapitulating key dynamic features of the developing human brain at the molecular, cellular, and structural levels	[18]
	hESCs and hiPSCs	Combined biopolymer scaffold and Matrigel embedded	Developmental study and disease model	The cerebral organoid derived by guided cortical plate formation using a biopolymer scaffold showed a distinctive radial organization of the cerebral cortex and allow for the study of neuronal migration.	[19]
	hESCs	Matrigel embedded	Transplantation model	Organoid grafts showed a progressive neural maturation and regeneration, followed by neovascularization when transplanted into mouse	[20]
	hiPSCs	Orbital suspension culture and guided bioprinting	Drug screening and regenerative therapy	Patterned vascularized cortical organoid containing neural stem cells, endothelial, and neurons that mimic the vascularized brain shows its potential in drug testing and regeneration	[21]
	hiPSCs	V-bottomed 96-well plate	Drug Screening and disease modeling	Cerebral organoids demonstrate the utility of this organoid system for modeling the teratogenic effects of Zika virus infection as well as testing the therapeutic compounds that can mitigate its destructive actions	[22]
	hESCs	U-bottom ultra-low-attachment 96-well plate	Disease modeling	The presence of vasculature-like structures resulted in enhanced functional cortical organoids maturation for disease modeling applications	[23]
	hESCs	ultra-low-attachment 96-well plate	Disease modeling	The 3D system creates the reciprocal projections between the thalamus and cortex by fusing the two distinct region-specific organoids that represent the developing thalamus or cortex.	[24]
Oesophagus	hESCs and hiPSCs	Matrigel embedded	Disease modeling	Human esophageal organoids were utilized for modeling the human esophageal birth defects, with enhanced survival.	[25]
Thyroid	hESC	Hanging drop	Transplantation model	hESC-derived thyroid follicles that produce thyroid hormone in vitro and in vivo after transplantation into thyroid gland ablated mice.	[26]
Thymus	hESCs and hiPSCs	Static transwell-6 well plates	Transplantation model	The thymic organoids was validated for in vitro generation of antigen-specific T cells	[27]
Heart	hiPSCs	Micropatterned	Drug screening	The cardiac organoid was successfully representing a decent in vitro pro regenerative drug development and potential reduction of their adverse effects	[6]

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	hiPSCs and hESCs	U shape 96 well and matrigel-embedded	Gene targeting and Drug testing.	In this genetic defects in vitro study, heart-forming organoids represent the simultaneous monitoring of effects caused by genetic mutations, as well as drug screening of teratogenic substances	[28], [29]
	hiPSCs	2D micropatterned	Developmental study and drug-induced cardiac developmental toxicity.	The development of cardiac organoids presented a platform of comprehensive risk-assessment cardiac developmental toxicity assay which better predicts thalidomide toxicity on fetal health.	[30]
	hiPSCs	3D micropatterning using PDMS molds and poles	Disease modeling and regeneration	human cardiac organoids have been proposed as the physiologically relevant model of the human heart and completely recover the cardiac function after cryoinjury	[31]
	hiPSC	3D micropattern by agarose hydrogel molds	Drug screening	The cardiac organoids recapitulate 3D tissue-level responses including drug-induced/exacerbated cardiotoxicity and fibrotic effects. This organoid can be applied for studying doxorubicin induced-myocardial infarction	[32]
Lung	hESCs and hiPSCs	Matrigel embedded	Transplantation model	Human PSCs-derived lung bud tip organoid was able to be engrafted into the airways of the mouse model	[33]
Liver	hiPSCs	Suspension culture and matrigel embedded	Disease modeling	Hepatic organoid consisting of hepatocytes and cholangiocytes was successfully utilized for the disease modeling of liver genetic disease	[34]
	hESCs and hiPSCs	Embedding inside the matrigel dome	Drug screening and disease modeling	Several drug screening that was retracted from the market, such as troglitazone, trovafloxacin, and levofloxacin, was successfully employed using liver organoids. Additionally, this organoid was also utilized for modeling hepatic steatosis.	[35]
	hESCs and hiPSCs	Dynamic suspension culture in Erlenmeyer flask using an orbital shaker	Transplantation, drug screening, and disease modeling	The functional vascularized liver organoid presents with liver-like functional features, which included the production of serum proteins and the coagulation factors, as well as supported ureagenesis and bilirubin uptake.	[7]
	hESCs and hiPSCs	Induced pluripotent stem cells	Disease modeling and drug screening	The hepatobiliary organoid organized a functional bile canaliculi system, which was disrupted by cholestasis-inducing drugs.	[36]
	hiPCs	Combined static microwell culture, 24-well, 6-well, and 1-well plate.	Transplantation	Vascularized and functional liver organoids generated entirely from iPSCs significantly improved hepatic functionalization properties and represent functional rescue against acute liver failure via transplantation.	[37]
pancreatic duct-like organoids (PDLOs)	hiPSCs	Microwell chip	Disease modeling	pancreatic duct-like organoids expressed either mucins or the cystic fibrosis transmembrane conductance regulator.	[38]
Blood vessel	iPSC	Coculture techniques in static ultra-low attachment six-well plate	Transplantation model, Modelling diabetic vasculopathy, Drug screening	Transplanted blood vessel organoids are grown in streptozotocin-treated mice represent a model of diabetic vasculopathy and its drug treatment.	[39]
Kidney organoids	hiPSCs	Ultra-low-attachment 6 well plates followed by spinner flask biorector	Disease modeling and drug screening	The kidney organoid was successfully modeled the congenital kidney defect that interferes with tubulogenesis and potential drug treatment.	[40], [41]
Assembloid (multiple organoid fusion)	hiPSCs	Matrigel embedded	Transplantation model	Transplantation of gut and liver organoids showed an effective therapeutic potential against fulminant liver failure	[42]
	hPSCs	96 well-round bottom low attachment plates	Disease modeling, drug development, and therapeutic transplantation.	Human hepato-biliary-pancreatic organoids showed therapeutic responses to new potential treatments, modeling biliary atresia and promoting pancreatic fate commitment in Hes1-deficient mice.	[43], [44]

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Direct organoid differentiation from hPSCs EBs

Several approaches were previously developed to generate the hPSCs derived-organoid as a miniaturized specific tissue or organ. The simplest strategy is to directly induce the differentiation toward organoid formation from the three-dimensional structure of hPSCs, called embryoid bodies (EBs). This structure can be cultured in dynamic suspension culture, hanging drop techniques, or embedded in Matrigel (Figure 1). Afterward, the EBs can be treated by a stepwise treatment using a cocktail of biomolecule inducers which mostly consisted of several signaling factors (e.g., small molecule and/or growth factors) which mimic the cellular signaling during organogenesis in embryo development. This approach permits intrinsic self-organization during directed differentiation which generates further three-dimensional structures consisting of several cell lineages which comprised a specific organ [45]. However, the chance of the generation of undesirable cell types which not complemented with the expected organ was more difficult to control during differentiation.

Coculture of multiple differentiated cells

Direct organoid differentiation from the hiPSCs EBs usually resulted in problematic cell heterogeneity from undesirable differentiated cell types, making the reproducibility of resulted organoid identity often becoming low. To address this issue, the hPSCs can be differentiated separately, sorted, and rearrange towards organoid. This technique can be a solution for the uncontrollable heterogeneous cell population. The hPSCs-derived cellular components can be achieved by performing an in vitro co-culture of multiple PSCs-derived cells or PSCs-derived progenitor cells (Figure 1). First, the specific progenitor cell type was individually differentiated from PSCs using monolayer culture. Afterward, each cell component is purified and co-cultured with other cell components required to construct a certain organoid type. Since the organoid is assembled from the independent sorted cell type from the hPSCs, the occurrence of other cells which are not normally part of the expected organs can be significantly minimized. To improve cellular diversity inside the organoids, these specific cell types can be added based on the actual ratio of the cellular composition of the original organ. This mixed heterogeneous aggregate will form an organoid structure which expected to be more represent the morphological features and physiological functions of the specific organoid type. Moreover, the structure can be further improved by some engineering approaches, such as 3D cell bioprinting or scaffold based-template to partially direct the cellular arrangement inside the structure.

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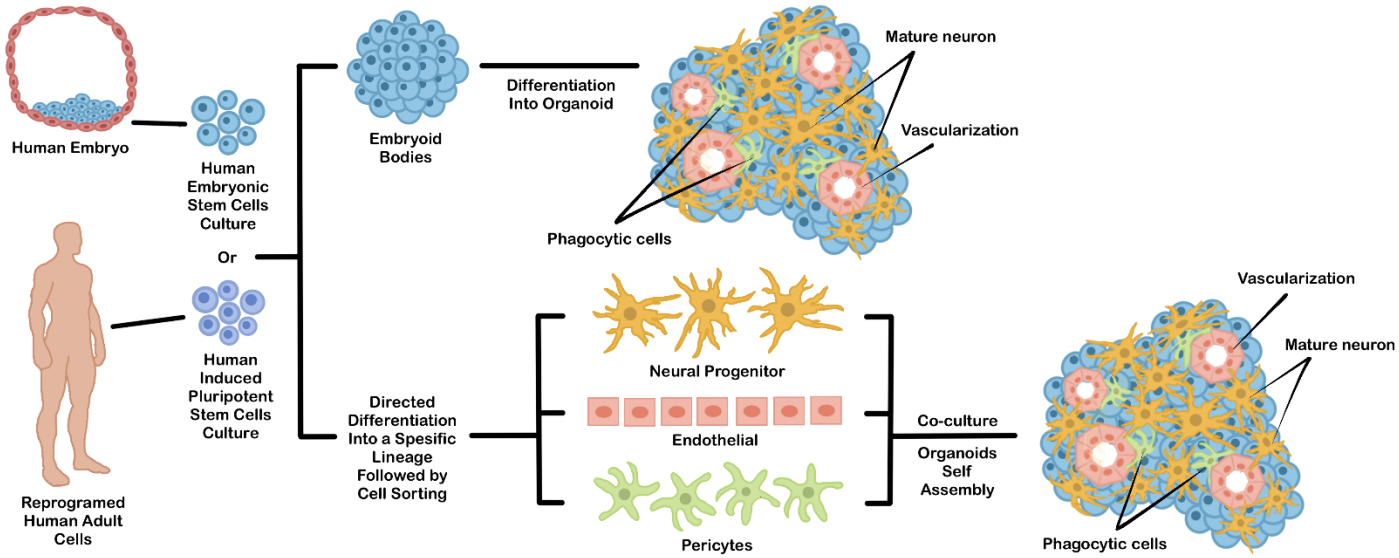

Figure 1. The example of general strategies to generate hPSCs derived-brain organoids. This organoid can be generated directly from differentiated EBs (upper chart) or by coculture from independently differentiated hPSCs derived-cell types (lower chart).

4. General culture platform to generate the hPSCs derived-Organoids

Matrigel embedded technique

As similar as organogenesis in vivo, the extracellular matrix (ECM) remodeling plays an essential role during organoid morphogenesis, mainly for the branching formation that dictates the functional architecture of certain organs, such as the salivary gland, lung, or kidney [46]. Matrigel is an extracellular matrix extracted from Engelbreth-Holm-Swarm mouse sarcomas which are routinely used in various cell culture applications [47]. In conventional monolayer culture of hPSC, the Matrigel acts similarly as a feeder layer which is supporting its proliferation and pluripotency. During the organoid differentiation, the Matrigel dome plays a role as an extracellular matrix (ECM) which serves as structural support and provides a three-dimensional niche for the organoid assembly[47]–[50] (Figure 2A).

Despite its benefits in organoid culture, Matrigel also has some disadvantages. The variations in biochemical composition and mechanical variability of this sarcoma-derived-ECM complex may result in a high single- and batch-to-batch variability during the hPSCs derived-organoids culture [51]. Additionally, since organoid technology was recently shifting toward transplantation, the usage of xenogeneic materials needs to be eliminated. These materials from animal-derived components such as Matrigel or animal-derived-serum also may limit the therapeutic application of hPSC-derived organoids. In actual therapeutic applications in humans, this animal-origin substance may potentially induce the antigenicity and transmit a pathogen into hPSCs cultures, such as viruses[52].

Recently, several efforts were developed to find synthetic alternatives for the Matrigel [51], [53], [54]. These alternative materials may provide a chemically defined and xeno-free culture niche that can be independently tuned by adjusting various variables, such as its composition and the polymerization method to produce the expected mechanical and biological properties [51], [53]. Moreover, by using a bioengineering approach, these synthetic matrices can be further utilized to provide a specific boundary guiding tissue morphogenesis [55].

Another challenge of the matrigel embedded method is related to its application in organoid-based transplantation of large organs. Due to its technical difficulties in scaling up, this technique may be limited to drug screening and disease modeling.

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Static suspension culture

A static suspension culture can be used as an alternative to producing a broad spectrum of quantities in organoid production, from a small scale (e.g. patterned well plates or hanging drop method), medium-scale (e.g. low attachment culture dish [56], [57]), or larger scale (e.g. oxygen-permeable static tissue culture bag [58]) (Figure 2B). This method can be selected when the usage of ECM embedded is not required and when the interference of hydrodynamic condition need to be avoided to generate a specific lineage that sensitive to shear stress.

Traditionally, the early formation of the three-dimensional structure relies on the spontaneous aggregation in the static suspension culture. This simple method was adapted to generate the hPSCs derived-organoids in a medium or large-scale organoid production, for example using a low attachment culture dish [56], [57] or culture bag [58]. The main drawback of this technique is the failure to control the EBs aggregation due to the epithelial characteristic of hPSCs that induced a spontaneous aggregation, resulted in a random size, impacting the variability of their 3D differentiated organoid structure. Therefore, several alternative methods have been developed to improve the aggregation control, such as the hanging drop method or utilizing a patterned culture vessel (Figure 2B). The hanging drop method is a technique to induce cellular assembly by utilizing gravitational force. The cell suspension droplet is hung on the reversed surface, and the EBs formed from the surface tension properties of the culture medium. This method is relatively simple during cell inoculation, but the medium replacement is very challenging due to the limited droplet volume that can be hung on the culture vessel surface [59]. Patterned well plates (e.g. microwell plates, U or V shaped-well plates) can be used as a tool to force and control the cell aggregation [60] [14]. Combined with the adjustment of cell number, this system enables more precise control of the EBs size, which significantly reduces the variability of resulting organoids.

Dynamic suspension culture

Dynamic suspension culture can be achieved by rotating or stirring mechanisms to prevent the cell adhesion to the culture vessel surface and promote the formation of a spherical 3D formation, called spheroid through aggregation. Several platforms that are routinely used for organoid generation are by putting the well plate in a rotational shaker or stirred bioreactor (Figure 2C). These types of suspension culture enable better control of spheroid size and uniformity in a large amount at once, which influences the dynamic of hiPSCs expression factors of pluripotency and their differentiated counterparts [61]. The spheroid size and uniformity can be controlled by adjusting the inoculation density and manipulating the medium dynamics, such as rotation/stirring speed, fluidic flow, or medium volume [52], [62].

Hydrodynamic condition is an important biomechanical factor in the dynamic suspension culture that may affect the differentiation process of hPSCs. Despite its importance in controlling the agglomeration and enhancing medium mixing in suspension culture, the amount of hydrodynamic shear force needs to be carefully considered. Our previous comparison study showed that the diverse hydrodynamic condition coming from the shear force in various culture vessels set up, such as rotational culture using culture dish and 6 well plates, ring-shaped culture vessel, or spinner flask may implicate the differentiation tendencies of hPSCs. The results indicated that higher shear stress improved the differentiation tendency of hPSC EBs into ectoderm and mesoderm lineage, rather than endoderm [61]. A study conducted by Wolfe, et al. revealed that the shear force induced early germ specification into ectodermal and mesodermal lineage on stress magnitude, ranging from 1.5 to 15 dynes cm^{-2} [63]. This result also corresponds with an-

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other study conducted by Vosough et al. which reports a number of spontaneously differentiated-mesodermal populations from hepatic differentiation which is presumably exposed by excessive shear stress in the stirred tank bioreactor [64].

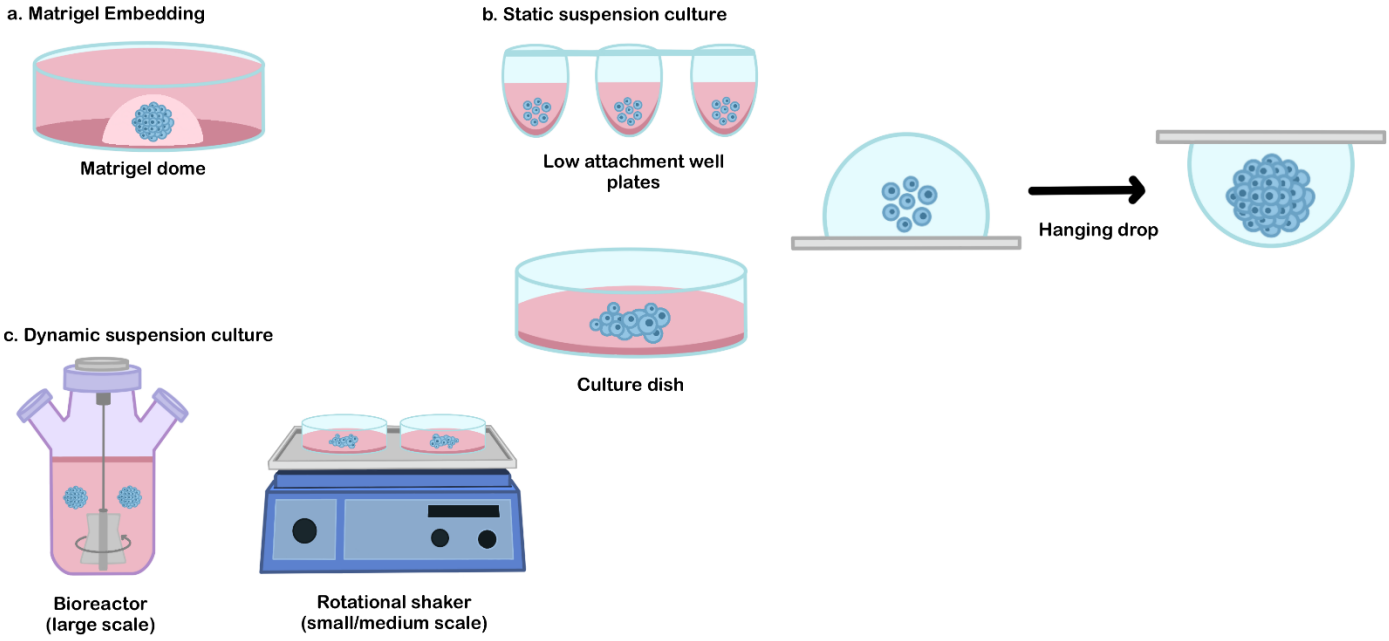

Figure 2. The common culture platform for the hPSCs derived-organoid. (a). Matrigel embedding technique, (b). Static suspension culture, and (c). Dynamic suspension culture

5. Other Limitations and Possible Improvements of PSCs Derived-Organoids Culture

Maturation

Currently, a number of methods for generating the hPSCs derived-organoid were reported the broad spectrum of a specific tissue functionality, such as nephron filtration [40], beating cardiac organoid [65], insulin-secreting organoid [66], or organoid with drug metabolism capability [35]. However, the efficient techniques to obtain a mature and functional organoid remain challenging [41]. The PSCs derived-cells are still relatively immature and exhibit the expression profile of fetal- or progenitor cells, rather than adult tissue cell type [40]. Moreover, these cells still lacking in both architectural and functional characteristics compared to their original adult tissue counterparts. These maturation problems not only impact their reliability in disease modeling or drug screening but also importantly affect the graft immaturity and safety concerns when they are used in regenerative therapy [67]

A functional organ usually takes several years to be fully mature in vivo [68]. However, current studies in hiPScs derived-organoid generation mostly performed the culture only within a couple of weeks to several months. Based on this understanding, hypothetically, extending the culture period using a differentiation induction medium may improve their physiological and structural maturation. Giandomenico and his colleagues showed a good example of this approach to achieve further maturation [69]. In the prolonged maturation period, they have successfully generated cerebral organoids with a telencephalic identity of later stages of neural development, including axon outgrowth and neuronal maturation [69]. Another study also showed a similar result in hPSC derived-cardiac organoids [70]. The extended culture up to 50 days display improved cardiac specification, survival, and maturation, as well as response to cardioactive drugs.

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Table 2. A brief comparison of the common culture platform for organoid production

Consideration	Animal model	hPSCs differentiation or coculture in monolayer	hPSCs derived-organoid		
			Matrigel Embedded /dome	Static suspension culture	Dynamic suspension culture
Culture maintenance	Not required, but difficult animal handling	Easy	Relatively easy	Relatively easy	Relatively easy
Represent actual human organ`s physiology	Partial, limited by interspecies variability	Poor	good	good	Good
Tissue complexity	Very good	Poor	good	good	Good
ECM and cell-cell interaction	Very good, native tissue condition	Poor	good	good	Good
Ability to represent organogenesis/developmental biology	No	No	Yes	Yes	Yes
Represent actual human organs physiology	Partial, limited by interspecies variability	Poor	good	good	Good
Scalability	Not available	Low	Low	High	High
Relative cost production	high	Relatively high	Relatively low	low	Low
Application for personalized medicine and non-xenogeneic transplantation	Not possible	possible	Not possible	possible	Possible

The signaling pathways play a vital role in regulating the mature phenotypes during organ development and growth. This mechanism involved several biomolecules, such as cytokines, growth factors, hormones, inhibitory molecules, and other small molecules. By using the screening method, we may identify the specific regulator and optimize the in vitro organoid maturation using the same molecule compositions [71], [72]. An experimental approach conducted by Huang et al, uncover the potential of this method [73]. A combination of biochemical factors, such as thyroid hormone, dexamethasone, and insulin-like growth factor-1 (TDI) has been shown to improve the maturation of hiPSC-derived organoids [73]. Besides using exogenous signaling molecules in the culture medium, they also can be provided by coculturing the hPSCs-derived cells with other cell types that existed in the original organs. These additional cell types may endogenously secrete molecule components required to promote further maturation of hPSC-derived-organoids. One example showed by a study to incorporate endothelial and mesenchymal stem cells that secrete a specific factor that induced further maturation in hPSCs-derived kidney organoids [74].

Metabolism is a critical process that influences self-renewal and cell fate specification of hPSCs [75]–[77]. Based on this knowledge, switching the energy source in the culture medium may direct the cellular metabolic adaptation towards a more matured and functional cell type. For example, the increasing concentration of glucose and an adjustment of palmitate concentration in the culture medium can significantly improve the hPSCs derived-cardiac organoid maturation [78].

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To be fully matured, the hPSCs-derived-organoids required a complex signal that partially can be mimicked in vitro. However, the in vivo niche can provide better and more complete necessary signals similar to their developmental or maturation period. For instance, Dye et al. found that in vivo transplanted hPSCs derived-lung organoids were showing improved cellular differentiation of secretory lineages and resulting in airway-like structures that were remarkably similar to the native adult human lung.

High-cost production

Different from disease modeling and drug screening purposes, clinical/translational regenerative therapy applications are often required a decent amount of cell numbers. For instance, a billion cells are necessary to regenerate both partial and total organs, such as the heart, pancreas, or liver [79]–[81]. Therefore, the scalability needs to be performed by robust biomanufacturing processes which involved a high-cost production. Among all of the culture components, the cost consumption is mainly comes from the requirement of expensive recombinant proteins, such as growth factors, that essentially required to induce the differentiation of PSCs into a specific and functional cell type.

Some effort has been conducted to minimize the requirements of these components, such as the development of growth factors-free differentiation protocols using the small molecules [82]. However, the differentiated cells often showed a less functional maturation compared to the ones differentiated by the inclusion of the growth factors. Since the usage of cytokine or growth factors still becoming a necessity, an alternative solution is developed to optimize the usage of growth factors efficiency during differentiation. For example, using a specific molecular weight cutoff dialysis membrane barrier to recycle and accumulate the high molecular weight endogenous and exogenous growth factors while continuously maintaining the exchange between the small molecular weight nutrition and toxic metabolic byproducts [83]–[86]. This approach may significantly improve the production efficiency per unit of hPSCs-derived organoids.

Tissue complexity

As a model system, hPSCs-derived organoids still show a partial in vivo features of the original human tissue, such as complete tissue architecture, functional immune regulation, innervation, and vascularization. Therefore, this miniaturized structure is often failed to imitate a complex and integrated interplay between each organ system.

During the hPSCs derived-organoids culture, several progenitor cells developed from hPSCs are communicating via paracrine factors to arrange the tissue formation in vitro. This self-organization was not only affected by their microenvironment and cellular interaction inside the structure but also by their culture environment, which is normally difficult to control and resulted in variability in the same or between batches [87]. A number of attempts have been conducted to precisely define the structural tissue-like geometry by niche modification [30], which also potentially reduces batch-to-batch variability during organoid construct. For example, a bioengineering approach called “guided self-organization” which utilized a fiber scaffold to control the organoid elongation was reported [19]. This technique shows an improvement in increasing the tissue complexity, enhanced differentiation, and better reproducibility.

In the actual human tissue, vascularization plays a vital role in exchange of various necessary components for tissue homeostases, such as nutrition, oxygen, and signaling molecules. The absence of this vasculature in organoids was becoming one of the major limitations in the current development of organoid technology. During in vitro culture period, the organoid may excessively grow up to a millimeter in size. This condition causes a limited passive diffusion of nutrients, oxygen, signaling molecules, and toxic cellular byproduct inside the structure which potentially creates a gradient of important molecules diffusion. The failure to control this spatiotemporal distribution of these molecules

may lead to the development of necrotic tissue inside their three-dimensional structure[60], [61].

Although the organoid vascularization can be achieved during in vivo tissue engraftment through native neovascularization in vivo [88], [89], the generation of the vascular structure during in vitro culture remained difficult. In order to address this problem, several vascularization techniques can be performed to generate vascularized organoids. A simple method to enable vascularization is by performing a co-culture between the predifferentiated hPSCs or hPSC derived-progenitor cells with endothelial cells before or during the aggregation period to let them self-organized. The earlier development of this method was introduced by Takebe et al. [90], [91] by introducing endothelial cells (HUVECs) and mesenchymal stem cells (MSCs) into hPSCs derived-multipotent hepatic endoderm spheroid to generate a vascularized liver buds organoids. A similar technique was also performed by Pettinato et al. [57] by interlaced human adipose microvascular endothelial cells (HAMEC) within the hiPSCs spheroids, followed by hepatic differentiation. Alternatively, the mesoderm can be incorporated into the EBs and they will differentiate into endothelial cells to form a vascular network [92]

To further improved the vascularization inside the organoid, the bioengineering approach can be applied using 3D fabrication techniques. A recent study by Skylar-Scott et al [21] showed a bioengineering approach to producing a vascularized organoid by controlling the composition and structure using tissue bioprinting. In this technique, the hPSCs derived-progenitor cells were individually incorporated within hydrogel and printed using a layer-by-layer deposition to form the expected structure [93]–[95]. Another strategy to enable vascularization is by utilizing a sacrificial networks method using a degradable- or removable biomaterial. The vascularization was formed by seeding endothelial cells or hPSC-derived-endothelial cells in the space created by the voids from the removal of sacrificial networks [95]. Although all of these 3D biofabrication techniques provide an enormous potential to guide the assembling of 3D organoids, several challenges still need to be addressed. In addition to the poor survival of hPSCs or hPSC-derived progenitor cells, they are very sensitive to environmental cues due to their embryonic-like nature in response to the developmental signal. Moreover, they also tend to spontaneously agglomerate and forming a random clusters after being printed [93].

The other components that often lacking in current hPSCs-derived organoids to mimic the actual organ are the neuronal innervation complex and resident immune system. Neuronal innervation is an essential part of an organ that regulates various biological processes. For example, acetylcholine (ACh) is a neurotransmitter that triggers cholinergic signaling which plays a pivotal role in the regulation of the small intestine cells [96]. A study conducted by Workman et al. tried to provide the solution to this problem by introducing the hPSCs derived-neural crest cells (NCC) to hPSCs-derived intestinal organoids [97]. Inside the organoid structure, the ECC has shown migration and formed a microglia structure. This complex structure showed a functional regulation of intestinal contraction and can be utilized to investigate the mechanism of motility disorders of the human gastrointestinal tract [97].

To demonstrate the immune regulation for developmental study and disease modeling application, several important immune components need to exist in organoid culture. Several attempts have been carried out to mimic the conditions by adding some cytokines to partially imitate the in vivo immune regulation [98]. However, this approach did not completely reflect the complete regulation by immune cells since they also secrete several cytokine complexes [99]. Moreover, the immune cells also play a critical role during infection, such as phagocytosis [100]. An experimental approach was conducted by Xu et al. to investigate the possibility of immune cell inclusion by incorporating hPSC-derived microglia into brain organoids [17]. The results showed that the hPSCs derived-microglia exhibit phagocytic activity and synaptic pruning function inside the cerebral organoid. Additionally, the secretion of neurotrophic factors and cytokines, such as insulin growth factor 1 (IGF1) and IL-1b was able to modulate the neurogenesis in organoid structure [17].

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Most of the current studies are concentrated on the single hPSCs derived-organoid type. To enable a study about a complex interaction between different organs, a few studies have constructed multi-organ organoids or assembloid by fused and interconnecting multiple organoid types that exhibit a specific function, for example, hepato-biliary-pancreatic organoids [43], [44] or retinoganglial organoids [101]. Although the organ crosstalk can be simulated, this approach was still limited to the utilization of early progenitor organoid types which does not completely reflect the fully mature and functional organ. Another problem that requires more investigation is the method to precisely control further maturation of each organ after fusion since each organoid type required specific growth factors and small molecules cocktail, as well as different culture environment conditions to be fully mature.

5. Conclusion and Future Perspectives

Organoid technology is a remarkable technology that may provide the ex vivo platform of miniaturized organs for various applications, such as in vitro model of organogenesis, disease modeling, drug screening, and further possibilities to be applied for regenerative therapy. To realize the best utilization of the hiPSCs derived-organoids, the selection of the culture system needs to be carefully considered, depending on the types of application and the effect culture environment during the culture period. Additionally, despite their improvement by the bioengineering approach, several challenges still need to be addressed to ensure their reliability in reconstituting the actual complex organ function and their safety for future regenerative therapy.

Optimizing detailed variables and mechanisms related to the culture condition during organoid forming and differentiation from hPSCs into each specific organoid type. This approach also can be improved by integrating some engineering approaches such as the utilization of biocompatible material and 3D bioprinting. By using these optimizations, a standardized methodology can be developed for generating organ-specific- hiPSCs derived-organoid.

The personalized hiPSCs-derived organoid not only can overcome the individual variability problems in drug screening and disease modeling but also enable immune compatible graft for full- or partial organ transplantation when combined with xeno-free organoid production.

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